Applicants: Graham P. Allaway et al.
Serial No.: 09/888,938
Filed: June 25, 2001
Exhibit 7

PCT WORLD INTELLECTUAL PROPERTY ORGANIZATION
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INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 5 : A61K 31/47, 31/645 — A1

(11) International Publication Number: WO 92/01451

(43) International Publication Date: 6 February 1992 (06.02.92)

(21) International Application Number: PCT/US91/03383

(22) International Filing Date: 15 May 1991 (15.05.91)

(30) Priority data:
536,887 20 July 1990 (20.07.90) US

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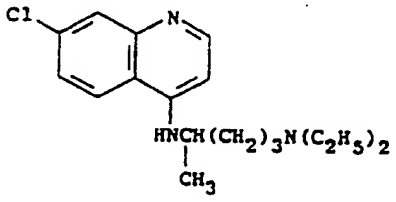
(81) Designated States: AT, AT (European patent), AU, BB, BE (European patent), BF (OAPI patent), BG, BJ (OAPI patent), BR, CA, CF (OAPI patent), CG (OAPI patent), CH, CH (European patent), CI (OAPI patent), CM (OAPI patent), DE, DE (European patent), DK, DK (European patent), ES, ES (European patent), FI, FR (European patent), GA (OAPI patent), GB, GB (European patent), GR (European patent), HU, IT (European patent), JP, KP, KR, LK, LU, LU (European patent), MC, MG, ML (OAPI patent), MR (OAPI patent), MW, NL, NL (European patent), NO, PL, RO, SD, SE, SE + (European patent), SN (OAPI patent), SU, TD (OAPI patent), TG (OAPI patent).

Published
With international search report.

(54) Title: METHOD FOR KILLING HIV-INFECTED CELLS



(57) Abstract

The present disclosure demonstrates the use of chloroquine to selectively kill HIV-infected cells. The invention involves, in part, the use of chloroquine in treatment regimens for the treatment of HIV-infected individuals, wherein chloroquine is administered to such individuals in pharmacologically effective doses. While it is proposed that chloroquine may be administered to HIV-infected individuals alone, it is believed that additional benefits may be realized through the administration of chloroquine in combination with other agents such as anti-HIV immunotoxins (e.g., CD4-based immunotoxins, or anti-gp41 or anti-gp120-based immunotoxins). In these embodiments, it is believed that chloroquine will act synergistically with the anti-HIV immunotoxins to effect a selective killing of HIV-infected cells. While the use of chloroquine is preferred, it is proposed that other related compounds such as 4-aminoquinoline antimalarials or chloroquine [illegible] may also be employed to provide benefits in accordance with the invention.

+ DESIGNATIONS OF "SU"

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METHOD FOR KILLING HIV-INFECTED CELLS

The government may own certain rights to the present invention pursuant to NIH grant AI-27336.

The present invention relates to a method of treating Human Immunodeficiency Virus (HIV) infections, wherein chloroquine is employed to selectively kill HIV-infected cells.

HIV is a retrovirus that causes a variety of immunological deficiencies in humans. Particularly, it causes acquired immune deficiency syndrome (AIDS) and AIDS related complex (ARC).[1] In AIDS, the HIV virus debilitates the body's immunological defenses, mainly by infecting and killing T4 ($CD4^+$) lymphocytes and releasing immunosuppressive gp120 from infected cells. T4 lymphocytes defend against invading foreign matter; thus, their destruction renders the body susceptible to a spectrum of diseases. In addition to infecting T4 lymphocytes, HIV also infects other immune system tissue such as monocytes, nervous system tissue, intestinal tissue and probably some bone marrow cells. Monocytes and macrophages (matured monocytes) are not killed outright by HIV. Rather, it is believed that, once infected, these cells serve to continuously incubate the virus.

Cells that are susceptible to HIV infection typically have on their surface a molecule known as T4 (CD4). A specific HIV protein known as gp120, whose precursor is gp160, recognizes and binds to CD4. Once binding has occurred, core proteins and viral RNA of the virus are delivered into the cell. The viral RNA is then transcribed into DNA by a reverse transcriptase. This DNA can then

integrate into the host cell's DNA (in which case the infection is latent) or it can commandeer the cell's biochemical machinery for viral replication. If replication is rapid enough, the host cell lyses.

Treatment of HIV infections poses particularly formidable challenges because HIV infects the very cells that defend against infections. Furthermore, the simplicity of retroviruses, their propensity to alter their antigenic properties, and their ability to integrate into the host cell's genome have troubled researchers trying to design specific treatments. Additionally, treatment of HIV related central nervous system (CNS) disorders, is complicated by the blood-brain barrier's general impenetrability to drugs.

With an infection rate rapidly rising nationally and globally, HIV is becoming a leading cause of death. Currently, no particularly efficacious treatment for HIV infections has been identified. What is available is generally nonefficacious, highly toxic and nonspecific. Some positive results have been achieved by treating infected individuals with azidothymidine (AZT), which presently is the principal FDA-approved treatment for AIDS. AZT is believed to be a reverse-transcriptase inhibitor and a terminator of RNA or DNA extension. AZT therapy, however, does not cure the infection; it only prolongs survival time and ameliorates some of the disease's symptoms. Furthermore, treatments are costly.

AZT treatment has many drawbacks. For example, the drug is highly toxic to many individuals. Although AZT does appear to improve the quality of life, there is no indication that AZT actually is effective in all circumstances. It certainly does not cure the disease. Some individuals are particularly sensitive to AZT and cannot tolerate it.

In addition to AZT, there are other drugs on the horizon, however, these are merely in the clinical trial stage. For example, substances such as phosphonoformate, rifabutin, ddC, ddA and dd, may inhibit HIV's reverse-transcriptase. Some of these may also act as chain terminators of RNA and DNA chain extensions. Other substances, such as dextran sulfate, rCD4 and certain monoclonal antibody products, may serve to inhibit viral binding at CD4 sites.

These agents also suffer from significant side effects and drawbacks. The methods, such as rCD4 and dextran sulfate, are of questionable efficacy. Therefore, there is currently a great need for an agent low in toxicity that is effective, simple to use, and relatively inexpensive. The present invention meets these qualifications. Chloroquine selectively kills HIV-infected cells, and yet has little or no side effects or toxicities, and, therefore, can be well tolerated.

Although chloroquine has been studied as a method for treating HIV infections, its use has been discounted because it is thought only to inhibit viral entry into cells or inhibit replications of virus and infected cells. Accordingly, there is still a great need for methods which also treat infected cells. Particularly, there is a need for methods that will kill HIV-infected cells as opposed to methods that are simply directed to either alleviating the symptoms or inhibiting replications.

An object of this invention is to selectively kill cells infected by the Human Immunodeficiency Virus. Another object is to provide a drug that is useful in the treatment of HIV infections whose general pharmacology is known and predictable. Yet another object is to provide a treatment that is relatively low in cost when compared with currently available treatments such as AZT.

Chloroquine's pharmacology as to humans is well known to persons skilled in the medical sciences. Advantageously, chloroquine concentrates in the same tissues that HIV is believed to infect. The inventor's studies reveal that chloroquine is toxic to HIV-infected human cultured cell lines. Accordingly, it is expected that chloroquine will safely and effectively treat human HIV infections such as AIDS, ARC, and HIV-related central nervous system disorders.

Methods of administering chloroquine to HIV-infected individuals are expected to be similar to how it is administered in treating human malarial infections. These include oral or parenteral administration. The invention is carried out by subjecting cells infected by the HIV virus to a sufficient amount of chloroquine to kill HIV-infected cells. The HIV infected cells can be killed by subjecting them to a concentration of 10 to 20 μg/ml/day of chloroquine. However, a preferred concentration range is 2 to 5 μg/ml/per day of chloroquine. It is contemplated that this method can be adapted for killing HIV infected cells in humans. In the practice of an embodiment of the invention, a dose of 300 to 500 mg per day of chloroquine per day is administered to individuals infected with the HIV virus. Administration is continued until HIV infected cells are killed. In treating infected individuals, it is proposed that chloroquine can be administered orally at a dose of 300 to 500 mg per day, or, alternatively, parenterally at a dose of 200 to 300 mg per day. Chloroquine can also be used to treat the symptoms of debilitation, chronic opportunistic infection, and autoimmune reactions, which are associated with HIV infections. In treating symptoms, chloroquine is administered at a dosage sufficient to reduce one or more of the symptoms.

In treating symptoms, administration of chloroquine can, again, be orally at a dose of 300 to 500 mg per day or parenterally at a dose of 200 to 300 mg per day. In either case, this administration is continued for the duration of the infection. In treating the infection or the symptoms, the administration of the above dosages can be adapted to include an initial dose which is double the dosage of the subsequent doses.

Chloroquine may also be combined with an immunotoxin having anti-HIV activity such as a CD4-, anti-gp120- or anti-gp41-based immunotoxin, preferably linked with ricin A chain or deglycosylated ricin A chain (dgA), for enhanced toxicity to the infected cells. In embodiments where chloroquine is combined with an immunotoxin, the dose will typically be between about 200 and 300 mg of chloroquine, and between about 30 and 50 mg of the immunotoxin, administered over a one week course.

Figure 1 shows the effect of varying concentrations of chloroquine on cultured U937 human cells infected with either a SAN (•) or BAG (O) HIV isolate, or on uninfected control cells of the same kind (▲).

Figure 2 shows the effect of various concentrations of immunotoxins in combination with a constant concentration of chloroquine and without chloroquine on HIV-infected cultured human cells and uninfected cells of the same kind. In Figure 2A is shown the effects of treatment with either control immunotoxin (Ig-dgA, ■) or with anti-gp41 immunotoxins (antibody 98-6-dgA, O; antibody 50-69-dgA, ▲) on viability of HIV infected H9 cells. Shown in Figure 2B, are the effects of these agents on control, uninfected H9 cells. In Figure 2C is shown a similar study, excert for the inclusion of chloroquine at a concentration of 2×10^{-5} M, together with the respective control or anti-gp41 immunotoxins (open symbols), and the use of HTLV III_b

infected U937 cells. In Figure 2D is shown a control using these same agents on uninfected U937 cells.

Chloroquine, widely known as an anti-malarial compound, is also known to have certain antiviral effects.[2] It has previously been proposed that chloroquine can inhibit viral *entry* into cells. *In vitro* studies indicate that chloroquine prevents the processing of envelope glycoproteins involved in recognizing CD4.[3,4] Some *in vitro* studies also indicate that chloroquine inhibits pH-dependent entry of HIV into uninfected cells.[5]

The present invention is a breakthrough, beyond these known effects of chloroquine, in that chloroquine is employed to selectively kill cells already infiltrated by HIV. In *in vitro* IC_{50} tests, HIV-infected human H9 T-cells have been found by the inventors to be up to fifty-fold more sensitive to chloroquine than uninfected cells. Studies also indicate that the concentrations necessary to kill infected cells *in vitro* are within the dosage range used for *in vivo* treatments of other diseases such as malaria.[6,7] (See, *infra*, example 1). It is proposed that chloroquine functions in part by reducing the reservoir of HIV infected cells and thereby reduces or delays the progression of the disease.

In mammals, chloroquine collects in higher concentrations in some tissues than in others. Advantageously, the tissues where the higher concentrations of chloroquine are found include the tissues that HIV is most likely to infect. Advantageously, chloroquine concentrates in the lymphoid system, wherein HIV-infected T cells, monocytes, dendritic cells and B cells are believed to reside. These cells are major reservoirs of HIV in infected humans. Leukocytes also concentrate chloroquine. Additionally, chloroquine concentrates in the cerebrospinal fluid. Hence, the blood-brain barrier would not impede using

chloroquine to treat HIV infected CNS tissues. Chloroquine concentration in the liver, spleen, kidney and lungs can be 200 to 700 times greater than in the plasma.

Chloroquine's quality of specifically concentrating in the very tissues likely to be infected by HIV means that chloroquine can be delivered to these tissues in an appropriate dosage while exposing the tissues unsusceptible to infection to a relatively low dosage. This quality of chloroquine should minimize the potential for adverse reactions in the unsusceptible tissues.

It is further expected that chloroquine can be compatibly used in combination with other pharmacologicals. For example, *in vitro* studies show that chloroquine, in conjunction with immunotoxins, enhances the immunotoxin's effect. (See example 2) This is particularly true of immunotoxins directed against the gp41 component of HIV, but is also true of immunotoxins directed against gp120. Likewise it is contemplated that chloroquine will also synergize with other drugs such as AZT.

Chemically, chloroquine is a type of 4-aminoquinoline. It is proposed that other pharmacologic agents similar in structure to chloroquine, such as other members of the 4-aminoquinoline family or chloroquine family agents which have similar pharmacologic action to chloroquine, will find similar utility in accordance with the present invention. Exemplary congeners, generally of the 4-aminoquinoline family, include amodiaquine, hydroxychloroquine, quinacrine, pamaquine, pentaquine, and the like (see, e.g., Wiselogle[9]).

Chloroquine's general pharmacology is well studied from its use in treating malaria [6,7]. In addition to its anti-malarial properties, chloroquine has proved effective against a variety of other disorders, including

inflammatory diseases, certain lupus diseases, certain photoallergic reactions, and cardiac arrhythmias.

Chloroquine is believed to have several biological mechanisms: it completely inhibits certain enzymes, it has been shown to inhibit incorporation of ^{32}p-labeled phosphate into RNA and DNA, and it tightly binds to double stranded DNA. Chloroquine also inhibits DNA and RNA polymerase by combining with DNA primer.[7]

The metabolism of chloroquine is well understood. When ingested, chloroquine quickly and nearly completely assimilates into the bloodstream, binding mostly to nondiffusible plasma constituents. The body does not rapidly excrete chloroquine, but it does degrade chloroquine into several metabolites. Particularly, chloroquine is degraded into a metabolite called desethylchloroquine and bisdesethylchloroquine. Degradation metabolites account for about 30% of the chloroquine absorbed by the digestive system. The remaining 70% is unchanged chloroquine.

In accordance with the present invention, it is proposed that Chloroquine can be taken orally, in tablet form, or parenterally by injection. Orally, chloroquine is given either before or after meals. It is expected that chloroquine can be administered to treat HIV infections by selectively killing HIV infected human cells in an individual diagnosed with HIV.

It is proposed that the following administration and dosages may be employed to treat HIV infections. An initial dose of about 600 mg is administered, orally or parenterally on each of two consecutive days. Suppressive doses of about 300 mg daily should then be given weekly for 2 to 3 weeks. Infants or children should not be

administered more than about 10 mg of chloroquine per kilogram of body weight per day.

Chloroquine is known to have some side effects; these are discussed in detail in medical references such as Goodman and Gilman's, The Pharmacological Basis of Therapeutics and the Physician's Desk Reference. A brief description of some of chloroquine's known side effects follows.

Few significant long term effects are known even after prolonged administration of about one year. The side effects that do occur normally disappear with discontinuation of treatment. Known side effects of chloroquine include mild headaches, visual impairments, gastrointestinal upset, and pruritus. Because chloroquine concentrates in the liver, it should be cautiously used on patients suffering hepatic disease. It must also be cautiously used in the presence of gastrointestinal, neurological or blood disorders. Chloroquine should not be used with gold or phenylbutazone as it might produce or complicate dermatitis. Persons on long-term therapy should also be given periodic ophthalmological examinations. Finally, unless absolutely necessary, chloroquine should not be used during pregnancy.

The examples which follow demonstrate the use of chloroquine to selectively kill HIV-infected cells (Example 1), and the use of chloroquine in combination with anti-HIV immunotoxins, which together act additively in their anti-HIV effects (Example 2). As will be appreciated, these examples illustrate various embodiments for carrying out the invention. The studies set forth below were conducted in part through the application of standard laboratory practices of the inventors as well as procedures developed by the inventors or otherwise found to work well in the practice of the invention. Various modifications,

rearrangements of the steps, substitutions, and the like will be apparent to the skilled artisan in light of these examples. While these examples have been conducted using model systems in vitro, those of skill in the art will recognize that these model systems are exemplary and representative of in vivo treatment methods.

EXAMPLE 1

This example demonstrates the successful use of chloroquine to selectively kill HIV-infected U937 cells, an accepted test system for demonstrating anti-HIV activity. (See Figure 1). It also shows doses of chloroquine which have been successfully employed to kill HIV-infected cells.

U937 is a monocyte cell line that was selected because it can be chronically infected with HIV and is resistent to the lytic effects of the virus. The tested U937 cells comprised a set of cells infected with the BAG isolate of HIV, a set of cells infected with the SAN isolate of HIV, and uninfected cells. The BAG and SAN isolates are separate HIV isolates from different patients. The control cells were infected cells but were not subjected to any chloroquine. Serial dilutions of chloroquine were plated in 96-well microliter plates. Cells were then added to make a final concentration of 4×10^5/ml. After the plates were incubated for 36 hours they were pulsed with tritiated thymidine. Cells were then harvested and thymidine incorporation determined using a Beta counter. The uptake of tritiated thymidine in the cell's DNA gives a measure of cell division at a specific time.

Figure 1 shows the effect of increasing concentrations of chloroquine on the viability of identical cells handled identically except with and without exposure to chloroquine. The effects were measured by measuring the uptake of labeled thymidine, which is a measure of DNA

replication and, hence, cell viability. In Figure 1, the symbols represent: (○) U937 infected with the BAG isolate of HIV; (●) U937 infected with the SAN isolate of HIV; (▲) uninfected U937 cells. Results are expressed as a percentage of control cells.

Figure 1 shows that as the concentration of chloroquine was increased, so did the toxic effects that were observed against the HIV-infected cells. These studies show that HIV infected cells were fifty times more sensitive to chloroquine than uninfected cells. In other words, at a concentration of about 2×10^{-9} M, 50% of the U937 cells infected with either of the HIV isolates were killed, while the uninfected control U937 cells were unaffected. From Figure 1 it can also be observed that the effective concentrations at which infected cells are killed *in vitro* are within the range of doses used to treat other diseases *in vivo* (e.g. malaria).[6,7] The doses used in this study were about 1 to 20 μg/ml, and thus similar to dosage ranges that have been obtained for chloroquine in patients in the treatment of malaria.

EXAMPLE 2

This study was undertaken to demonstrate that combining immunotoxins with chloroquine results in greater toxicity to infected cells than either component would have against infected cells alone (See Figure 2). Immunotoxins are conjugates formed between a cell surface binding ligand, such as a cell surface-directed antibody, and a toxin moiety, such as deglycosylated ricin A chain (dgA). This study investigates the combined anti-HIV effects of chloroquine and anti-HIV immunotoxins prepared using antibodies directed against gp41. (gp41 is an envelope component of HIV[8]).

In this study, the concentration of the immunotoxin-dgA conjugate (IT-dga) employed was varied while keeping the concentration of chloroquine constant at 2 x 10^{-5} M. Each IT-dgA was plated in serial dilution. Dilutions were made in complete media (RPMI 1640 with 15% (v/v) heat inactivated fetal calf serum and antibiotics), with or without chloroquine. Cells were then added and cell viability measured by thymidine uptake as described in Example 1. The results are expressed in Figure 2 as viability in terms of percentage of control cells which remain alive (untreated cells ± chloroquine). The controls employed for these studies included the use of a polyclonal human immunoglobulin immunotoxin prepared by Sandoz, Inc. and having specificity unknown to the inventors (Ig-dgA), as well as the use of uninfected cells.

Figure 2A shows the effects of treatment with either control immunotoxin (Ig-dgA, ■) or with anti-gp41 immunotoxins (antibody 98-6-dgA, O; antibody 50-69-dgA, ▲) on viability of HIV infected H9 cells. As can be seen, with either of the anti-gp41 immunotoxins, a 50% killing of HIV infected cells was achieved at a IT-dgA concentration of between about 10^{-9} and about 10^{-10} M. In contrast, as shown in Figure 2B, control, uninfected H9 cells were unaffected by the IT-dgA treatment.

In Figure 2C is shown a similar study, except for the inclusion of chloroquine together with the respective control or anti-gp41 immunotoxins (open symbols), and the use of HTLV III_b infected U937 cells. As can be seen in Figure 2C, when the anti-gp41 immuntoxins were administered alone, a 50% killing of HTLV III_b infected U937 cells was observed at between about 10^{-8} and 10^{-9} M. However, when chloroquine was included, a dramatic shift in the curve of about 2 to 3 orders of magnitude to the left was observed, with a 50% killing at a concentration of between about 10^{-10} and about 10^{-11} M. As in the case of the anti-gp41

immunotoxins alone on uninfected cells (Figure 2B), no effect was observed for the combination of anti-gp41 immunotoxins with chloroquine on uninfected U937 cells.

These studies indicate that subjecting infected cells to a combination of chloroquine and IT-dgA results in significant toxicity to the HIV infected cells without significantly affecting uninfected cells.

The foregoing examples demonstrate studies performed by the present inventors in the development of the invention. It is believed that these examples include a disclosure of techniques which serve to both apprise the art of the practice of the invention and, additionally, serve to demonstrate its usefulness in various settings and to disseminate general knowledge which relates peripherally to more central aspects of the invention as defined by the appended claims. However, it will be appreciated by those of skill in the art that the techniques, compositions, and embodiments disclosed herein are preferred embodiments only and that in general, numerous equivalent methods and techniques may be employed to achieve the same or similar results.

The following references are specifically incorporated herein by reference to the extent that they disclose or teach methods or compositions useful in the practice of the invention, or provide background information useful in understanding the invention, particularly, broader aspects of the invention.

1. *See generally, What Science Knows About AIDS--A Single Topic Issue*, 259(4) Scientific American 1 (Oct. 1988).

2. E.g., Helenius, K., et al., *On Entry of Semliki Forest Virus into BHK-21 cells*. J. Cell Biol. Vol. 84, 404-420 (1980).

3. *Wiley, et al., Biosynthesis, Cleavage and Degradation of the Human Deficiency Virus-1 Envelope Glycoprotein, gp160,* 85 Proc. Nat'l Acad. Sci. (USA) 9580 (1988).

4. Maddon, P.J., *et al., The T4 Gene Encodes the AIDS Virus Receptor and is Expressed in the Immune System and the Brain,* 47 Cell 333 (1986).

5. Stein B., *pH-Independent HIV Entry into CD4-Positive T-Cells Via Virus Envelope Fusion to the Plasma Membrane,* Cell Vol. 49, 659-68 (1987).

6. *Physicians Desk Reference,* pp. 2237-40 (43 ed. 1989).

7. *The Pharmacological Basis of Therapeutics* (Goodman & Gilman 5th Ed. 1975).

8. see U.S. application serial numbers 155,336, filed 2/12/88, and 323,486, filed 3/14/89.

9. Wiselogle, F.Y. (ed.), *A Survey of Antimalarial Drugs, 1941-1945.* J.W. Edwards, Pub. Inc., Ann Arbor, Mich., 1946 (Two Volumes)

CLAIMS:

1. A method of killing HIV-infected cells, comprising subjecting said cells to a concentration of a 4-aminoquinoline antimalarial or chloroquine family agent sufficient to kill HIV-infected cells.

2. The method of claim 1 wherein the agent comprises chloroquine.

3. The method of claim 1 wherein the HIV-infected cells are subjected to a concentration of chloroquine of between about 1 and about 20 μg/ml.

4. The method of claim 2 wherein the HIV-infected cells are subjected to a concentration of about 10 μg chloroquine/ml.

5. A method for killing HIV-infected cells in humans comprising:

(a) administering to an individual diagnosed with HIV a dose of a 4-aminoquinoline antimalarial or chloroquine family agent effective to kill HIV-infected cells; and

(b) continuing the administration until HIV-infected cells are killed.

6. The method of claim 5, wherein the agent comprises chloroquine.

7. The method of claim 6, wherein between about 200 and about 500 mg of chloroquine is administered to the individual per day.

8. The method of claim 5 wherein chloroquine is administered at an initial dose which is double the subsequent doses.

9. The method of claim 5 wherein chloroquine is administered orally at a dose of between about 300 and about 500 mg per day.

10. The method of claim 5 wherein chloroquine is administered parenterally at a dose of between about 200 and about 300 mg per day.

11. A method of treating symptoms debilitation, opportunistic infection or autoimmune disorder, which may be associated with HIV infections, comprising administering to an individual having such symptoms an amount of a 4-aminoquinoline antimalarial or chloroquine family agent that is effective to reduce one or more of such symptoms.

12. The method of claim 9, wherein the agent comprises chloroquine.

13. The method of claim 10 wherein the chloroquine is administered at an initial dose of double the subsequent dose.

14. The method of claim 10 wherein the chloroquine is administered orally at a dose of between about 200 and about 500 mg per day for the duration of the infection.

15. The method of claim 10 wherein the chloroquine is administered parenterally at a dose of about 200 to about 300 mg/day for the duration of the infection.

FIG.1

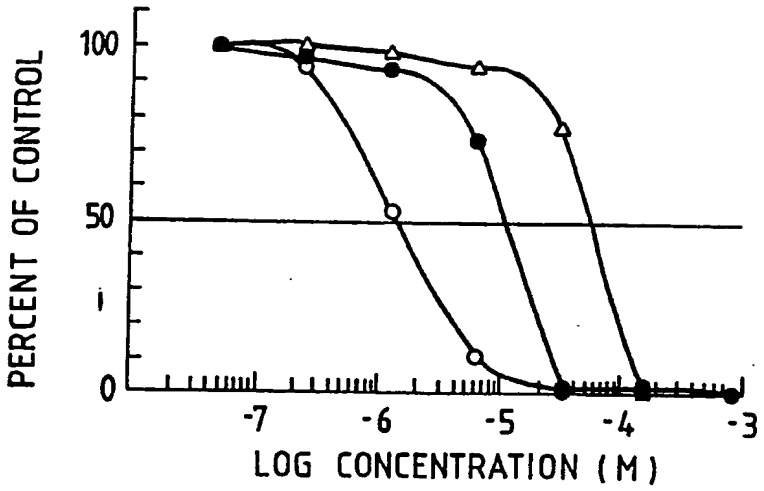

FIG.2A

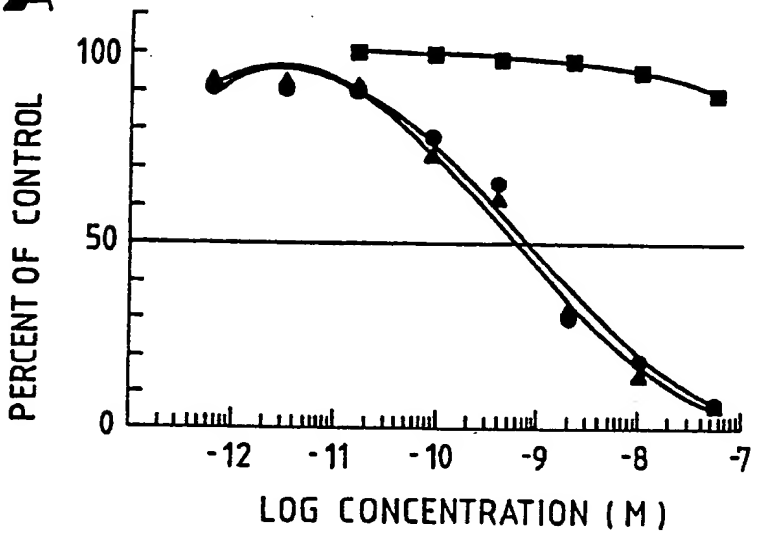

FIG.2B

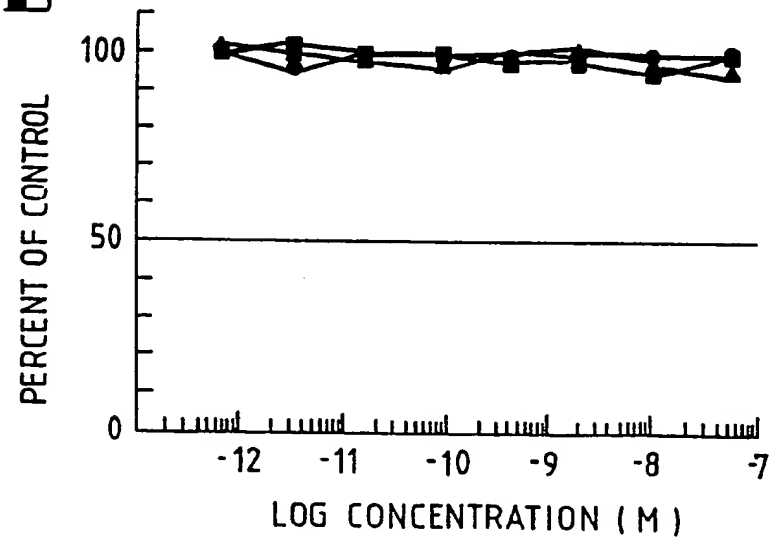

FIG.2C

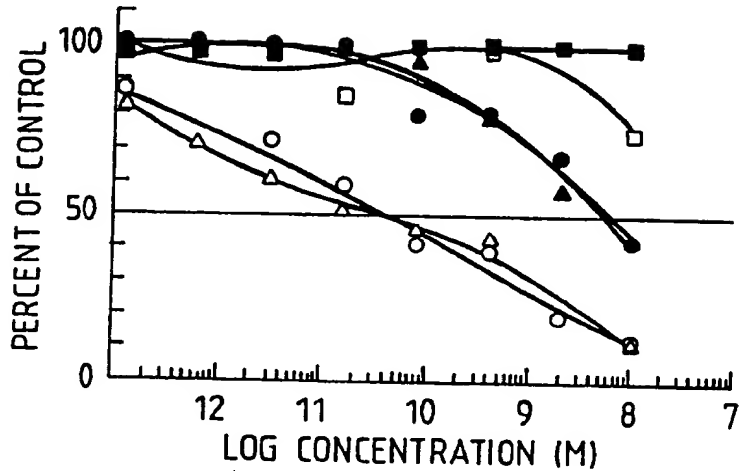
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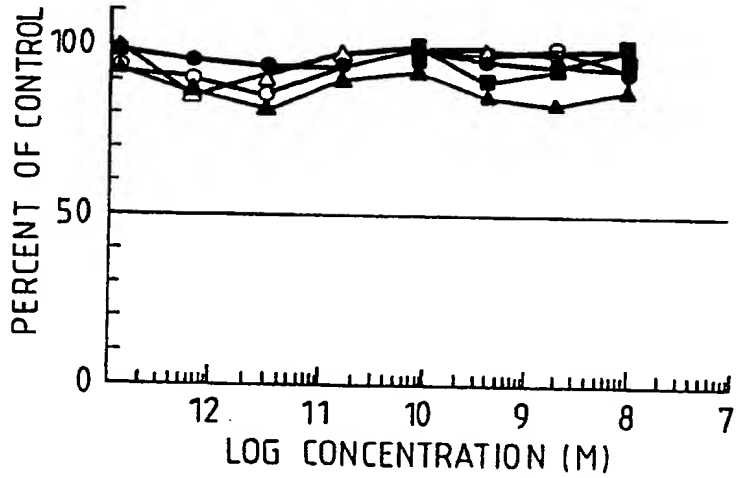
FIG.2D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International Application No PCT/US 91/03383

I. CLASSIFICATION OF SUBJECT MATTER (If several classification symbols apply, indicate all)[6]

According to International Patent Classification (IPC) or to both National Classification and IPC

Int.Cl.5 A 61 K 31/47 A 61 K 31/645

II. FIELDS SEARCHED

Minimum Documentation Searched[7]

Classification System	Classification Symbols
Int.Cl.5	A 61 K

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched[8]

III. DOCUMENTS CONSIDERED TO BE RELEVANT[9]

Category °	Citation of Document, [11] with indication, where appropriate, of the relevant passages [12]	Relevant to Claim No.[13]
X	Int. Conf. AIDS, vol. 5, 4-9 June 1989, abstract no. M.C.P. 119, I. Desportes et al.: "Effect of chloroquine on HIV1 infection of monocytes" ---	1-4
X	AIDS Research and Human Retroviruses, vol. 6, no. 4, April 1990, Mary Ann Liebert, Inc., Publishers, W.-P. Tsai et al.: "Inhibition of human immunodeficiency virus infectivity by chloroquine", pages 481-489 ---	1-4
X	Int. Conf. AIDS, vol. 5, 4-9 June 1989, abstract no. M.C.P. 66, W.P. Tsai et al.: "Inhibition of human immunodeficiency virus infectivity by chloroquine" ---	1-4
X	The Western Journal of Medicine, vol. 146, no. 2, February 1987, B.L. Kagan: "Lysosomotropic agents in AIDS treatment", page 234 --- -/-	1-4

° Special categories of cited documents : [10]

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report
27-08-1991	08. 10. 91
International Searching Authority	**Signature of Authorized Officer**
EUROPEAN PATENT OFFICE	Nuria TORIBIO

III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET)

Category °	Citation of Document, with indication, where appropriate, of the relevant passages	Relevant to Claim No.
E	Chemical Abstracts, vol. 114, no. 3, 21 January 1991, (Columbus, Ohio, US), & US-A-470 692 (NATIONAL INSTITUTES OF HEALTH) 1 August 1990	1-4

FURTHER INFORMATION CONTINUED FROM THE SECOND SHEET

V. [X] OBSERVATION WHERE CERTAIN CLAIMS WERE FOUND UNSEARCHABLE [1]

This International search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claim numbers 5-15 because they relate to subject matter not required to be searched by this Authority, namely:

see PCT-Rule 39.1(iv)

2. [X] Claim numbers (1-4)* because they relate to parts of the International application that do not comply with the prescribed requirements to such an extent that no meaningful International search can be carried out, specifically:

In order to avoid subject-matter excluded from patentability the method of claims 1-4 has been understood as an in-vitro method

*- partially searched

3. [ ] Claim numbers because they are dependent claims and are not drafted in accordance with the second and third sentences of PCT Rule 6.4(a)

VI. [ ] OBSERVATIONS WHERE UNITY OF INVENTION IS LACKING [2]

This International Searching Authority found multiple inventions in this International application as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this International search report covers all searchable claims of the International application

2. [ ] As only some of the required additional search fees were timely paid by the applicant, this International search report covers only those claims of the International application for which fees were paid, specifically claims:

3. [ ] No required additional search fees were timely paid by the applicant. Consequently, this International search report is restricted to the invention first mentioned in the claims; it is covered by claim numbers:

4. [ ] As all searchable claims could be searched without effort justifying an additional fee, the International Searching Authority did not invite payment of any additional fee.

Remark on Protest

[ ] The additional search fees were accompanied by applicant's protest.

[ ] No protest accompanied the payment of additional search fees